

CLAIMS

1. A method of detecting and identifying an orthopoxvirus within a sample comprising:

5 adding to the sample reagents for nucleic acid amplification and at least one pair of primers capable of amplifying at least one region of the orthopoxvirus genome, said region of the orthopoxvirus genome selected from the group consisting of HA and crmB;

incubating the sample under conditions suitable for nucleic acid amplification thereby producing an amplicon if the sample contains orthopoxvirus;

10 adding at least one restriction enzyme selected from the group consisting of: Sau 3AI, Spe I, Dra I, Hpa I, Ssp I, Alw 44I, Nla III, and combinations thereof; and determining if restriction enzyme digestion of an amplicon has occurred.

2. The method according to claim 1 wherein restriction enzyme digestion of an amplicon is determined by gel electrophoresis.

3. The method according to claim 1 wherein the pair of primers comprise 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2).

4. The method according to claim 1 wherein the pair of primers comprise 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

5. A pair of primers for detecting orthopoxvirus in a sample comprising 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2) or 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

6. A kit for detecting and identifying orthopoxvirus comprising:

30 at least one pair of primers selected from the group consisting of 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2); and 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

35 7. The kit according to claim 6 including at least one restriction enzyme selected from the group consisting of: Sau 3AI, Spe I, Dra I, Hpa I, Ssp I, Alw 44I and

combinations thereof.

8. The kit according to claim 6 including at least one positive control DNA template capable of binding to both primers of at least one primer pair.